PHYSIOLOGICAL PROPERTIES OF CHOLECYSTOKININ CONCENTRATES

BY

ROBERT WEBSTER DENTON

B.S., Northwestern University, 1943
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AN ABSTRACT OF A THESIS

SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHYSIOLOGY IN THE GRADUATE COLLEGE AT THE CHICAGO PROFESSIONAL COLLEGES OF THE UNIVERSITY OF ILLINOIS, 1950

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Introduction

This thesis is concerned with an attempt to evaluate the general physiological and pharmacological properties of the gastrointestinal hormone, cholecystokinin (CCK). Much of the work resolved itself into acquiring and interpreting a sufficiently large amount of data concerning the action of CCK concentrates upon the smooth musculature of the viscera. In addition, some reference to the interrelationships between CCK and other hormones as well as drugs is made.

CCK was defined experimentally in 1928 by Ivy and Oldberg (182) as the activity found in certain extracts of mammalian upper intestinal mucosa which elicits a contraction of the gall bladder when injected intravenously in the test animal. It is also considered the humoral agent naturally secreted by the small bowel in response to acid introduced into the lumen of the intestine which caused the gall bladder to contract. Most samples of secretin concentrates (first described by Bayliss and Starling (25)) also contain CCK activity; also secretin is present in CCK concentrates. Mellanby (237, 265) described cholecystokinin properties in his secretin preparations. Claims for a large number of hormonal moieties have been described in the literature as being present in mucosal extracts of the intestines, like enterocrinin, villikinin, enterocin, splenocontractile substance, etc.

In this paper we wish to identify our mucosal extracts as CCK concentrates by virtue of their ability to contract the gall bladder, and whatever other actions these preparations have are additional physiological properties or side reactions of the one hormone classification rather than their being a mixture of hormones, each exhibiting a separate property.

The preparation of CCK is relatively secure and its assay fairly reliable (130). A study of CCK might provide a model for the understanding of the more difficultly produced and assayed proteinaceous hormones as pituitrin and insulin.

The studies on our CCK concentrates were undertaken on both *in vitro* (as muscle strips) and *in vivo* (as balloon studies on the dog) experiments involving various sources and examples of smooth muscle of the viscera. CCK elicited contractions similar to those by histamine and acetylcholine. The CCK concentrates were antagonized by pituitrin, adrenalin, adrenal cortical extract, and insulin; they were somewhat antagonized by physostigmine and prostigmine, and not by atropine.

The literature contains several reviews on the gastrointestinal hormones (55, 88, 183, 233, 307, 289, 270). Still (372) summarized the pertinent literature up to 1931, and those of Greengard (138) and Grossman (142) constitute the most recent reviews.

The contributions to the subject of cholecystokinin, at least up to 1928, were mainly those concerning the effects of secretin preparations. The principal early references on the subject were made by Claude Bernard (29), Pavlov (311), Bekker (27), Dolinski (84), Popielski (315, 327), Wertheimer and LePage (408, 409, 407, 410) and Bayliss and Starling (25). In 1902 Bayliss and Starling demonstrated the idea of the hormone, secretin, which was liberated from the intestinal mucosa by acid introduced into the lumen of the upper bowel and which caused the pancreas to secrete. Within the next thirty years, investigations on the subject were directed along two lines of endeavor: first, the preparation of more specific vasodilatin-free concentrates was sought with an ultimate goal in view of obtaining crystalline material; and secondly, transplantation experiments insuring complete denervations of both the intestine and pancreas demonstrated the humoral effect of secretin. Similar transplantations of the intestine and gall bladder (182, 173) showed the humoral action of CCK.

The pertinent steps concerning the first problem are that Bayliss and Starling (25) used a 0.4% hydrochloric acid wash of ground-up mucosa; Wertheimer and LePage (407) used an acid made of mucosa not macerated; Weaver, Luckhardt, and Koch (404) precipitated the activity in a relatively stable form from the acid wash of Wertheimer and LePage by saturation with sodium chloride; and Ivy, Kloster, Drewyer, and Lueth (180, 189) extracted the salt cake with 70% ethanol, and after having evaporated off the alcohol, the activity (as the S-I) was precipitated with trichloracetic acid. Ivy (139) also introduced isoelectric precipitation in the procedure. Other work included mercuric chloride precipitation by Dale and Laidlaw (68), alcohol extraction by Stepp (370, 371), Agren (150, 5) and Mellanby (254, 275), bile salt precipitation by Mellanby, ultrafiltration of a regenerated picric acid precipitate by Takacs (377, 378, 379), and chloroform interface precipitation by Hammarsten, Wilander, and Agren (150, 5). Refining procedures using analine and picrolonic acid precipitations of the S-I were carried out by Hammarsten (372) and Agren (4) on an electrodialized concencentrate and by Greengard and Ivy (139). The crystalline secretin was found (141-B) to be principally pyridine, the secretin moiety being amorphous.

Investigations demonstrating the humoral nature of the product were those of denervations of an intestinal loop (save for sectioning the sym-

pathetics along the mesenteric vessels) by Bayliss and Sterling (25), transfusion of blood from a stimulated animal by Fleig (115), cross-circulation experiments by Matsuo (271), and finally transplantation and denervation experiments by Ivy and Farrell (185, 186) and Houssay and Mollinelli (172).

After Ivy described cholecystokinin in 1928 (182), investigations centered around the separation of CCK activity from the secretin activity in the secretin type of concentrates. Mellanby (276) described CCK activity in his secretin preparations, as have subsequent researchers (7, 87, 191). Mucosal extracts have been obtained with lower secretin unitage or with more enriched CCK potency (212, 180, 86, 7). Early references with crude secretin samples (302, 303, 46) noted contractions of the gall bladder. Ivy *et al.* (180, 187, 188, 189, 182) prepared secretin concentrates that rather consistently contracted the gall bladder following intravenous injection. By the use of cross-circulation and transplantation experiments (182, 173) similar to the ones advanced for proving the authenticity of secretin as a hormone, Ivy established the existence of a gall-bladder-contracting hormone which he called cholecystokinin. The four points of the hypothesis that cholecystokinin exists are: (1) dilute acid, fat, or an acid meal instilled into the upper intestine caused the gall bladder to contract; (2) none of these stimulants or their digestion products affected the gall bladder when they were injected intravenously; (3) acid instillation into a denervated (Thiry) loop caused contraction of an auto-transplanted gall bladder; and (4) certain extracts of the intestinal mucosa elicited gall bladder contractions upon intravenous injection.

Certain secretin preparations were also reported (273, 120, 276, 111, 138, 191, 31, 236) to elicit contractions in the small bowel; though much of the data is cursory (191, 120) or obtained on early crude extracts (182, 372, 383, 103, 104, 262, 263). Also various intestinal extracts, prepared somewhat as secretin is, have been reported to cause a variety of unrelated effects. If one had all the moieties attributed to gut extracts in one sample, it would elicit pancreatic and hepatic (4, 276, 126, 215, 216, 385) hydrogogic (277) flow by secretin, gall bladder contraction by cholecystokinin (182), intestinal movements by enterocin (276, 111), intestinal secretion by enterocrinin (291, 292, 117, 118, 119, 354, 293, 114, 414), diuresis (308, 314, 15, 266, 214, 355), hypoglycemi (385, 217, 71), splenic contraction (123, 33, 111), increased red blood count (196, 197, 213, 129, 218, 220), even increased antibody formation (89), regulation of alkali reserve of the blood (412, 413), calcium blood level effects (8), blood pressure depression (195, 22, 327), gastric secretion (392) with pepsin (329, 385), Brunner's gland secretion (120, 1, 119, 116, 9), a defensive hydration (385) by increasing the serum globulin, and others

(385). However, Agren (4, 7) in 1934 claimed that few, if any, of the "side reactions" occurred with his secretin "picrolonate."

Somewhat more substantial work has been described (291, 292, 354, 293, 114, 414) on intestinal extracts prepared as secretin which elicit a flow of intestinal *juices* on intravenous injection, including secretion from Brunner's glands (9, 10, 119, 120).

The references that argued for and against the existence of the various physiological actions of different secretin preparations can be exhaustively discussed. However, the scope of this abstract is such that even a listing of the references involved would make the paper too involved. In fact, the thesis in its long form (pp 17-25) does only little more than list the references, pro and con, about these questionable and unrelated properties of the muscosal extracts.

The most accurate and reproducible assay methods for evaluating the potency of CCK concentrates (as well as secretin concentrates) was developed only recently by Gershbein, Wang, and Ivy (130) by comparing the amounts of unknown concentrates that yield the same response (contraction of a gall bladder) as a standard concentrate in a non-erratic dog with dosages yielding results somewhere in the middle of the dose-response curve.

In vitro muscle bath preparations have been used as effectors for studying motility moieties in secretin concentrates, using the ileum (191, 7, 87) as well as the gall bladder (273, 191). Secretin was reported (95) even to increase the working power of skeletal muscle along with a delay toward fatigue of it.

Literature that challenged the existence of CCK can be listed here. Popielski (315-327) championed the "conservative" point of view that no special humoral hypothesis was required for the explanation of the Bayliss and Starling findings. The fact that secretin extracts of that time were high in vasodilatins lent strong argument for his views. Parsons and Koch (309) noted in 1924 that histamine and secretin were similar but differed in that secretin was a better secretogogue and histamine was a better vasodepressor. Other sources of secretin-like concentrates obtained from plant (83, 403) and animal (317, 318) tissues or from skeletal muscle (120) as well as from liver, pancreas, or duodenum (56) spoke of a non-ubiquitousness of source and possibly of a nonspecificity of character of the active substance or substances. The existence of such varied sources was strenuously denied by Ivy (93) and others (4, 405, 278). Yet Krimberg's (210) muscle extract preparations behaved similarly to duodenal secretin on intravenous injection. Also, Haberlandt's heart hormone (147) and v. Euler's "substance P" (182) had properties similar to secretin and CCK concentrates. Even angiotonin

was reported (156) to stimulate contractions of both the isolated and *in vivo* gall bladder and intestines.

Agren (7) reported extensive experimental data showing the differences between CCK and secretin. Electrophoresis and heating (to 280° C.) experiments seemed to differentiate the two entities in his hands. Heated histidine seemed to give positive secretin effects.

The above listing of references casts some light on the problem concerning the authenticity of CCK. Some evidence points toward the existence of a peculiar entity; other notes speak for a nonspecific factor.

The thesis reiterates the effects of insulin on visceral smooth muscle motility concomitant with the hypoglycemia. The influence of the vagus nerve on gastric motility and on the other abdominal viscera was similarly reviewed. Since these effects are relatively well known generally, they need not be discussed again here.

A review of histamine and its effects has been made by Best and McHenry (30). It is of interest that histamine has been extracted by procedures not unlike those employed for the extraction of the gastrointestinal hormones, and although the distribution of the amine was universal (1, 30), an exceptional source was found in the gastrointestinal tract (21). Thus, histamine could conceivably contaminate samples of the gastrointestinal hormones, even if it was not an active component of the concentrates. Histamine can elicit pancreatic secretion (284) and gall bladder contraction (137), but the effect is attributed to the vasodilation by the drug (310). Adrenalin antagonizes the effect of histamine to the extent that the vasodepression of histamine can be compensated for in the body (406). Also arginine, histidine, and cysteine seem to be able to inhibit histamine (96, 211, 65, 236). Histamine has been reported to be toxic to the isolated heart (128, 428, 346, 304). Like insulin, the effects of histamine need not be reiterated extensively here.

The thesis discusses the pharmacological effects of drugs and hormones on visceral smooth muscle *in vivo* and *in vitro* as aids in accomplishing the techniques and evaluating the results of the experiments done with the CCK concentrates. Again, the references were assembled for convenience, but the facts are generally well known and somewhat superfluous to the main thesis.

Experimental

Part A. Materials and procedures.

(1) Preparation of CCK concentrates.

In general, the extracts were prepared by the method of Greengard and Ivy (139). The 0.4% HCl wash was saturated with NaCl, and the salt-cake ("A-precipitate") was extracted with 80% ethanol. The alcohol

was removed by vacuum distillation, the aqueous residue was brought to a pH of 5.3 to 5.6, steamed to coagulate larger protein components; and the contents were filtered while hot. The filtrate (E-I) was usually treated to a final concentration of 5% trichloracetic acid. The precipitate (S-I) was separated and dried with acetone and ether.

Modification of the above procedure included treating the E-I with aluminum hydroxide gel (429, 212) to enhance potency. Also a diminished secretin content could accrue by shaking the E-I with chloroform and treating the supernatant fluid with trichloracetic acid (429).

The S-I when fractionated with analine in aqueous acetone yielded at least a two-fold enrichment of CCK. After the acetone and excess analine were removed under vacuum, the aqueous residue was treated with a ten-fold excess of acetone. After standing for 16 to 24 hours in the refrigerator, the precipitate was removed and dried with acetone and ether. The concentrates (VII) were white, water soluble powders. A list of concentrates used was given in Table A.

(2) Drugs and Hormones used: Insulin (Lilly), "Depropanex" (Sharp and Dohme), Pituitrin (posterior lobe extract) (Parke-Davis), Thyroid (Squibb), Estrogen, "Micropellets, Progynon" (Schering), Amnestrogen (Squibb), Chorionic Gonadotropin (Armour), Adrenal Cortical Extract (Upjohn, Harrower), ACTH (Armour), Prolactan (Rare), Purified secretin (Gershbein, Krup (212)).

Various readily available common drugs whose actions were cholinergic, adrenergic, sympathetic blocking, antihistaminic, anticholinergic, barbituric, and anesthetic, such as acetylcholine, prostigmine, physostigmine, choline, mecholyl, pilocarpine, adrenalin, ephedrine, ergotamine, thephorin, neoantergan, benadryl, atropine, cocaine, morphine, procaine, dibenamine, curare (Intocostin, Squibb), picrotoxin, tetraethylammonium chloride (Etamon, Parke-Davis), nicotine, strychnine were obtained from the Department of Pharmacology. The dosages for *in vivo* approximated those of the materia medica; for *in vitro* work dilute solutions were used.

(3) The muscle bath apparatus was constructed of glass in the usual manner, warmed to 37.5°C. aerated, and U.S.P. Locke-Ringer's solution was used. The isolated tissues (ileum, uterus, gall bladder, stomach, and urinary bladder) were supported in the bath by a glass hook and suspended to a writing lever from which kymographic tracings were made.

In the technique for mounting the specimens, emphasis was made that they be handled only with instruments.

(4) Apparatus for *in vivo* motility studies included a balloon (introduced into the dog's viscus) which was connected to a recording tambour by a tube. The system was filled with water and the height of the

tambour was usually within 5 to 10 inches above the balloon in the viscus (gall bladder, stomach, small bowel, colon, urinary bladder, or uterus). For awake dogs, gastrostomies, ileostomies, and cholecystostomies were performed, and the balloon inserted.

(5) Surgical procedures — The thesis describes the general methods employed in the surgery of the animals — gastrostomies, jejunostomies, gall bladder preparations, extirpation of the endocrine glands (castration, ovariectomy, pancreatectomy, adrenalectomy, thyroidectomy, and hypophysectomy).

(6) Assay procedures — For secretin — Method of Gershbein, Wang, and Ivy (130) on the dog pancreas.

For CCK — *In vitro* — Comparisons of heights of contractions by muscle strips (gall bladder and ileum) with different samples of unknown with the standard concentrates. Examples of the *in vitro* assay were given in Table B.

In vivo — In the nembutalized dog the ratio of unknown to standard concentrates was made using the amount the gall bladder contracted as the indicator.

In vivo — The effect of CCK concentrates using cholecystography. The areas of the shadowgraphs were compared to judge the degree of evacuation of the gall bladders in the awake dogs.

Part B. The effect of CCK concentrates on smooth muscle *in vitro*. Tables I-A, I-B.

A large amount of data (Table I-A) was accumulated on the effect of 21 CCK concentrates on guinea pig smooth muscle preparations from 59 longitudinal and 37 circular small bowel mounts, 28 gall bladders, 12 urinary vesicals, 11 colons, 11 stomachs and 8 uteri. Qualitative data of contractions by CCK concentrates was shown in all of these examples of smooth muscle.

Data relative to the effect of CCK concentrates on isolated smooth muscle of species other than the guinea pigs were given in Table I-B. Longitudinal and circular sections of small intestine, urinary bladders, stomachs, colons, uteri, and seminal vesicals of 7 rabbits, 6 mice, 2 hamsters, 7 cats, 4 pups (3 days old), 2 monkeys, 1 dog, 1 human (uterus) and 5 frogs were presented. CCK concentrates elicited contractions in these muscles. The number of contractions, relaxations, and nil-responses were entered for each example. Twenty-two notes for specific phenomena accompanied the tables.

Table I-C showed the "poisoning" effects of CCK concentrates on 17 isolated, perfused rabbit and 3 guinea pig hearts, where missed beats, arrhythmias, and eventual blocking resulted.

Part C. The effects of hormones and drugs on smooth muscle contraction by CCK concentrates *in vitro*.

Tables II-A and II-B present data on the effect of drugs and hormones on the viscera of guinea pigs and of a number of other species, respectively. Dosage ranges were incorporated with the title of the drug.

Antagonism and ineffectiveness of a number of hormones and drugs given before or just after CCK administration were shown on Table II-C more clearly than in Tables I-B and II-B. A list of observations taken from protocols (examples of which appeared in Chart I), including such muscles as longitudinal and circular ileal mounts, gall bladder, and uterus. The list of 26 drugs used has already been noted.

From Table II-A, II-B, and II-C one may note that (1) Many drugs gave negative effects on CCK concentrates. (2) Insulin antagonism to CCK often was lost spontaneously even though the insulin was still present in the bath. (3) Large doses of insulin (40 to 160 units) were necessary to antagonize the effects of a few milligrams of CCK concentrates. Also insulin antagonized the contractions due to histamine. (4) Although adrenalin almost always antagonized CCK concentrates, it usually did not block histamine contractions. (5) Adrenal cortical extract and obstetrical pituitrin exhibited strong antagonism to CCK concentrates, as did ACTH to a minor degree. (6) Large doses (0.3 to 1.0 mg.) of histamine were required to elicit contractions of the rabbit ileum *in vitro*. (7) Benadryl antagonized the action of CCK as well as that of histamine. The drug seemed to affect the longitudinal muscles of the guinea pig ileum more than the circular muscles. (8) Ergotamine was not active on the isolated guinea pig viscera *per se*, but it seemed to enhance the action of CCK concentrates. (9) Pituitrin and CCK concentrates could be biologically "titrated" against each other. (10) Curare (Introcostrin) was not very consistent in paralyzing smooth muscle against the action of CCK concentrates, especially the crude ones.

Part D. A comparison of CCK concentrates with histamine and pituitrin by inactivation studies (*in vitro*).

This work was initiated with the idea that heat or alkali treatments might differentiate between these drugs by destroying the activity of one or two of these without impairing the action of the third. Table II-D exhibited these data. Heat (autoclaving for 2 hours) and alkali (5% to 10% NaOH for 2 hours) did not destroy the histamine solutions, but they did "inactivate" the CCK concentrates. The pituitrin was "inactivated" more effectively by heat than by alkali. These data might indicate that histamine and CCK are different substances.

PART E. *In vivo* studies.

Section 1. — The effect of CCK concentrates as well as hormones and drugs on smooth musculature (*in vivo*).

Qualitative data on intact dogs corresponded quite well with the *in vitro* experiments in so far as the general effects of CCK concentrates were concerned; though pituitrin behaved quite differently from its effect in the *in vitro* experiments. Most of the work outlined in this section was performed on the dog by use of balloon techniques, usually on nembutalized animals.

Table III was a composite of several types of qualitative data on the effects of CCK on the intact viscera of the dog, cat and rabbit. It endeavored to note the responses of organs to CCK concentrates as well as to other hormones and drugs on dogs. Balloon data were also presented for two depancreatectomized dogs. It might be noted that almost every viscus contracted at some period or other in response to an injection of CCK concentrates.

In order to reduce the number of artifacts due to the dogs' moving and becoming excited, many of the animals were blindfolded during which time they rested quietly even when they were injected or when the balloons were introduced. The dogs experienced little or no pain during the experiments.

Synergism and antagonism of the hormones and drugs with the CCK concentrates in the intact animal were not well marked because of the capacity of the body to excrete, detoxify, and compensate for the drugs. Thus, the effects of the drugs in Table III were mainly those of their primary effects, which were fairly well known. Fourteen notes on Table III and a sample protocol (Chart II) were presented. Some of the most pertinent of these notes may be summarized.

(1) Most of the CCK concentrates did not cause a depression of the blood pressure. When the systolic blood pressure did fall, it recovered within one minute or so, whereas the effect of the CCK concentrates continued to act for ten minutes or so.

(2) Biphasic responses occurred occasionally, a relaxation below the base line during recovery following a contraction by CCK.

(3) Quantitative evaluation by balloon experimentation was hazardous because the viscus (especially the gall bladder) would often fail to relax to the base line following the contractions.

(4) Usually 10 to 50 mg. (20 to 200 units) of most samples was the dose of the CCK concentrates used. Whenever the dose was adequate enough to contract the small bowel or the stomach, the gall bladder invariably contracted. Also, small dosages often contracted the gall bladder without affecting the ileum.

(5) Contractions of the stomach varied with the condition of the dog as well as with the particular CCK concentrate used. Awake dogs (See Table X) often gave a relaxation instead of a contraction of the stomach on injection of a sample; however, awake, vagotomized dogs seldom gave a gastric relaxation. The gall bladder and small bowel contracted in response to the CCK concentrates in the awake, nembutalized or vagotomized dogs.

(6) Insulin in the depancreatectomized dogs caused a relaxation of the jejunum and gall bladder, but gave 3 contractions and 3 relaxations of the stomach.

(7) Although pituitrin occasionally contracts the bowel *in vivo,* it relaxed isolated specimens.

(8) Adrenalin contracted the small bowel on intravenous injections, but autogomized the CCK concentrates *in vitro.*

Section 2. Quantitative data on the effect of CCK concentrates on canine gall bladders (Cholecystography).

Cholecystographic studies in which the skiagrams of the gall bladder were taken before and after the administration of CCK comprised the data of this section. Tables IV-A and IV-B gave the percentage evacuations of the dog gall bladders following single injections of a sample containing from 6 to 240 units of a refined concentrate (on 20 dogs) and from 80 to 420 units of a more crude preparation (S-I) (on 17 dogs), respectively. The effect of consecutive doses of CCK, usually leading to complete evacuations, was presented in Table IV-C on five dogs. From 38 to 59% evacuation occurred in normal dogs of both sexes following one injection.

Section 3. The effect of extirpation of endocrine glands on gall bladder sensitivity, and CCK occurrence in the duodenum.

A. Cholecystographic data. Table V listed the cholecystographic results obtained with dogs in which various endocrine glands (ovaries, testicles, pituitary, thyroid, and pancreas) were removed. The four depancreatectomized dogs were more refractory to the CCK concentrates; whereas the gall bladders of four out of six of the ovariectomized females evacuated quite readily as did the three thyroidectomized animals.

In Table VIII a summary of the data was presented: 37 control (normal) dogs gave an average contraction of 49% with an average of 126 units of CCK; 8 male castrates contracted 28% with 107 units; one hypophysectomized dog evacuated 27% with 144 units; 3 thyroidectomized dogs contracted completely with 139 units; and 4 depancreatized dogs contracted only 16% with 120 units.

B. The specially operated dogs in secretin and CCK assays. The dogs with the extirpated glands were set up for assays at the conclusion of the X-ray studies: 5 castrates, 5 spays, 3 thyroidectomized, 3 hypophysectomized, one depancreatectomized, and 2 adrenalectomized dogs gave acceptable values for secretin and CCK occurring in a number of S-I and VII concentrates, each sample of which had been repeatedly checked on normal dogs earlier. There was no change in sensitivity of the operated dogs to the concentrates.

C. Distribution of secretin and CCK in the small intestines of dogs with extirpated glands.

S-I concentrates were prepared from the upper bowel of dogs with extirpated glands when they were eventually sacrificed. The respective S-I fractions were assayed for their secretin and CCK concentrates, and the ratio of secretin to CCK was 1:1, as the normal distribution. (Table VI).

D. Inactivation of secretin and CCK by sera from dogs with extirpated glands.

Sera from a castrated dog, a thyroidectomized dog, and an ovariectomized dog were used to "inactivate" enzymatically a CCK concentrate (SE-4) sample. The rate of "inactivation" approximated that of normal sera.

Thus, the dogs with extirpated glands gave no significant differences in the amount of extractable concentrates in the bowel wall, and the amounts of enzymatic destruction by their sera from normal.

Diagrams of apparatus used and examples of kymographic tracings for both *in vitro* and *in vivo* contractions of smooth musculature were presented in the section following the summary.

Discussion

The objective of this investigation has been to study the general physiological and pharmacological properties of the hormone, cholecystokinin (CCK), as it exists in the best concentrates which may be prepared today. It must be realized, since we cannot use the pure hormone, that some of the effects observed may be due to impurities. This, however, has been true of the study of all hormones until the pure product has been isolated. Progress has been made despite such handicaps. And, in certain cases, it has been possible, because of the nature of the action of the hormone to detect its action in the presence of impurities.

In order to decrease the variability, the concentrates containing CCK

were prepared by a uniform method. Hence, the concentrates used in this study, though not pure, were much more uniform than the preparations used by the numerous authors who have reported that intestinal or secretin containing extracts cause the contraction of the smooth musculature of many different viscera.

The first question which seemed to be desirable to answer was: *Does the substance in intestinal extracts which possesses what is termed cholecystokinin activity also stimulate the smooth muscle of all abdominal viscera to contract?* Or, is what we now term cholecystokinin a substance which stimulates the smooth muscle of the abdominal viscera to contract?

It was decided to seek the answer to this question by using *in vivo* and *in vitro* methods of testing the response of smooth muscle to excitants. It was realized that the *in vitro* method of using isolated organ or strips of tissue sets up rather artificial conditions, though the tissue in being separated from a nerve and blood supply may be considered as being under a better state of control by the experimenter. The *in vivo* method is complicated by the fact that a response to a stimulating agent is subject to the influence of compensatory processes.

The results on the *in vivo* and *in vitro* tests are summarized in Table XI. The results in general show that the CCK concentrate stimulates contraction of the smooth muscle of the gall bladder, jejunum, stomach, colon, urinary bladder and uterus.

This rather clearly demonstrates that CCK concentrates contain a smooth muscle excitant for the smooth muscle of the hollow abdominal viscera and that the gall bladder *in vivo* of the dog is the most responsive whereas *in vitro* there is not much difference in the responsiveness of the gall bladder, small intestines, and colon.

The fact that the intestine contracts with a frequency equal to or approximating that of the gall bladder was also observed by Sandblom *et al.* (433). They found that the duodenum contracted with a frequency of 87% as compared to 100% for the gall bladder in the dog. Ferguson *et al.* (111) found that the spleen contracted in 70% of the tests in which a secretin-CCK concentrate was used. However, the CCK concentrate used by Lueth *et al.* (256) did not apparently cause the intestine to contract so frequently; nevertheless, they did observe some instances of contraction.

It naturally follows that if what is called cholecystokinin activity in these CCK concentrates is a general excitant of the smooth muscle of the alimentary tract, then it may be considered a general alimentary motor stimulant released by fats, partially digested proteins, and dilute acids, but not by carbohydrates. On the other hand, CCK activity may be specific for the gall bladder musculature, and a second substance may be

TABLE XI

SUMMARY OF RESPONSES TO CHOLECYSTOKININ CONCENTRATES *IN VIVO* AND *IN VITRO*

(From Tables I-A and III)

DOG *IN VIVO*	*Relax*	*No Change*	*Contraction*	*Total Tests*	*% Contraction*
Gall bladder	0	8	54	62	87
Jejunum	4	25	57	86	66
Stomach	6	19	26	51	51
Urinary bladder	0	6	4	10	40
Uterus	0	16	9	25	36
Colon	0	6	1	7	14
GUINEA PIG *IN VITRO*					
Gall bladder	0	49	186	235	79
Jejunal and ileal					
Longitudinal	0	55	343	398	86 } 74
Circular	0	108	131	239	55 }
Stomach	0	64	6	70	0.9
Urinary bladder	0	47	9	56	17
Uterus	0	11	16	27	59
Colon	2	17	51	70	73

The majority of the preparations used on the stomach were faulty. Better results were achieved later on a wire loop mount.

the cause of the contraction of the intestine, colon, stomach, urinary bladder, uterus, and spleen. The answer to this question would be at hand if one or both of the substances could be chemically identified. Since this is not possible, an indirect attack is required to determine whether one, two, or more substances are present in CCK concentrates which cause contraction of the smooth muscle of the abdominal viscera.

It should be recalled in this connection that the term enterocin or peristaltin has been used to refer to the motor excitant in extracts of intestinal mucosa which cause the intestinal musculature to contract, and that whereas the existence of cholecystokinin as a hormonal agency has been established by sound evidence crucial in nature such is not the case for enterocin. However, another way to state the question raised in the previous paragraph is: Is what we call CCK activity in intestinal extracts identical with enterocin activity or are the two activities due to different substances?

There are two substances present in tissue extracts which exert excitatory effects on smooth muscle. These are histamine and acetyl choline. The evidence which rules out these two substances will be summarized.

That the motor excitant of smooth muscle present in CCK concen-

trates is distinct from acetyl choline and allied cholinergic drugs is quite obvious from the results. To recapitulate, the main similarities are (1) both contract smooth musculature, and (2) adrenalin antagonizes the contractions elicited by each. The differences are (1) atropine does not block CCK activity as it does acetyl choline, and (2) pilocarpine and prostigmine often antagonize contractions due to CCK.

Histamine and CCK show definite similarities and differences in their behavior. Histamine simulates CCK concentrates in that (1) Both are blocked and antagonized by benadryl (2-5 mg. in 40 cc. bath). (2) Both give similar contractions and tonus to *in vitro* preparations, provided the dosages are comparable. (3) Both are antagonized by pituitrin and adrenalin. (4) Each is extracted from the bowel, the upper intestine giving the best yields of each. (5) Both are refractory to the application of atropine. (6) Insulin elicited some relaxation against the contractions of each. And (7) both "poison" the isolated rabbit heart similarly. Histamine differs from CCK concentrates in that (1) Histamine injections elicit side reactions that are not shared by CCK concentrates, such as a fall in the blood pressure, and flushing or vasodilation. (2) Histamine in large doses can contract a gall bladder in the presence of adrenalin and the concentrates seldom, if ever, do. (3) Rabbit ileum in Locke-Ringer's solution is very refractive to histamine, requiring at least a milligram of histamine to contract the muscle, whereas, the CCK concentrates cause a contraction with the usual minimal dose used for the guinea pig viscera. (4) Histamine seems to demonstrate little or no latent period on the contraction of isolated muscle preparations whereas CCK concentrates invariably do. (5) Longitudinal intestinal strips usually contract more rapidly with histamine than they do with CCK, but when comparable effective doses are given, the contractions are similar. Histamine and the concentrates give similar step-wise contractions on the isolated circular strips (Table I-D). (6) Histamine is not "inactivated" by heat or alkali (Table II-D) as are CCK concentrates.

Since it is clear that what is termed CCK activity is neither histamine nor a histamine-like substance* and is not acetyl choline or a parasympathomimetic substance, the evidence may be logically directed to determine whether CCK activity is the sole smooth muscle excitant in the CCK concentrates.

The evidence pertaining to this question will now be summarized. *First, in vivo* the gall bladder of the dog appears to be more sensitive to CCK concentrates than other viscera, but *in vitro* in the guinea pig no significant difference in sensitivity was found (Table XI). *Second,* when

* It is possible that histamine or an imadazole may be a portion of the molecule responsible for CCK activity or what is termed enterocin activity.

the unitage of CCK activity on the guinea pig gall bladder *in vitro* is compared with the enterocin activity on the guinea pig ileum *in vitro*, the results show that the unitage of the two is approximately equivalent (Table XI). *Third*, when the CCK concentrates were treated with heat and alkali, the CCK and enterocin activity were both lost or inhibited. *Fourth*, when the isolated gall bladder and intestine of a guinea pig are treated with a CCK concentrate and contract vigorously to form a sustained contraction at a plateau, the contraction of the gall bladder and ileal strip is likewise relaxed by (a) adrenal cortical extract, (b) adrenalin, (c) pituitrin, (d) pilocarpine, prostigmine, and insulin, listed in order of the potency of their relaxing effect. This relaxing effect or "antagonism" is very striking and dramatic, and atropine does not possess such a property whereas pilocarpine or prostigmine does. (The pharmacology or more intimate physiology of this phenomenon would make a very intriguing and stimulating investigation. The effect of cortisone should be tried.) The relaxing effect of all these substances may be "washed out", but the insulin effect tends to "wear out" within a few minutes without being "washed".

There are two other approaches which were thought of but not used or studied extensively: one involving treating the CCK concentrate with normal serum to ascertain whether CCK activity and enterocin activity are inactivated equally. The other consisted in ovariectomizing dogs and guinea pigs and testing the gall bladder and ileum for any change in sensitivity to a CCK concentrate. This is very tedious and time-consuming work and the evidence obtained is presented in Table V, VI and VII. This approach was not carried far enough to bear completely on the question at hand. But the apparent increased sensitivity of the gall bladder after ovariectomy indicates that the approach is worthy of following further. Moreover, the increased sensitivity is in line with the observation of Smith, Pomerane and Ivy (432) that progesterone decreases the sensitivity of the guinea pig gall bladder to CCK-concentrates. (Incidentally in this work it was found that the removal of the gonads, thyroid and pancreas in the dog did not significantly alter the content and ratio of secretin and CCK activity of the upper intestinal mucosa.)

Another approach which is worthy of pursuing is the use of "Depropanex" (Sharp and Dohme), a saline solution of a protein-free, nitrogenous fraction obtained from acid-alcohol treatment of mammalian pancreas claimed to be free from insulin, acetyl choline, and histamine. This product causes contraction of the isolated ileum and gall bladder of the guinea pig. It should be tried on the gall bladder of the dog *in vivo* and its properties compared with extract of skeletal muscle. This is of sig-

nificance because muscle extracts do not contain CCK activity, but an extract of the pancreas may because of its embryonic derivation.

The results of the studies on the large list of various drugs and hormonal agents show that the CCK and enterocin activity is quite specific to acid extracts of the upper intestine. However, further work should be done on the "substance P" of Euler because the literature is not at present clear on this matter, since Euler has said that he found the substance in a preparation containing CCK activity (105).

The observation on the isolated, perfused heart of the rabbit indicate that when the preparation is repeatedly dosed with 1-5 mg. CCK concentrates an inhibitory or "poisoning" effect results much like that of histamine. The interpretation and application of this result is conjectural.

The general indication of the evidence presented in this thesis is that what is generally recognized as CCK activity in concentrates of acid extracts of the upper intestinal mucosa is also an excitant of the smooth muscle of the intestine, stomach, colon, uterus, and urinary bladder, though *in vivo* observations on the dog indicate that the gall bladder musculature is more sensitive. The crucial question, however, is not definitely answered by the evidence obtained. Nevertheless, more evidence has been obtained on the question than previously and future lines of worthy investigation bearing on the question have been delineated.

Summary and Conclusions

I.

1. The CCK concentrates stimulate contraction of the gall bladder, jejunum, stomach, colon, urinary bladder and uterus *in vivo* and *in vitro*. The *in vivo* evidence indicates that the gall bladder is somewhat more responsive than the other organs.

2. The evidence clearly establishes that neither acetyl choline nor histamine is the substance in CCK concentrates which causes the contraction of the smooth muscle of the hollow abdominal viscera and spleen.

3. It seems certain that the motor excitant in the concentrates is either CCK or some other substance which has not been identified.

4. All the types of evidence obtained in this study indicate, but do not categorically establish, that the CCK activity and enterocin activity (or the general visceral smooth muscle stimulating activity) are identical.

If this should prove in final analysis to be true then the presence of dilute acid, fat, and partially digested protein in the intestine cause the release of a general motor excitant. This would tend to antagonize the effect of enterogastrone on the stomach as regards fat. (It would be of

interest to ascertain the effect of enterogastrone concentrates on the intestine and the gall bladder.)

In view of the results obtained in this study and the implication of the general conclusion stated above, further study should be directed toward ascertaining whether a motor hormone for the intestine exists.

II.

In the course of this study several interesting observations were made which warrant special mention.

1. When the isolated gall bladder or intestine is contracted in response to a CCK concentrate the addition of adrenal cortical extract (Armour or Upjohn), adrenalin, pituitrin, pilocarpine, prostigmine, and insulin in order of potency, causes relaxation. This is of special interest because relaxation is caused by such a diverse group of pharmacologically active substances, and atropine, for example, has no effect.

3. The removal of the gonads, thyroid, and pancreas in the dog did not significantly alter the content and ratio of secretin and cholecystokinin activity in acid extracts of the upper intestinal mucosa.

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